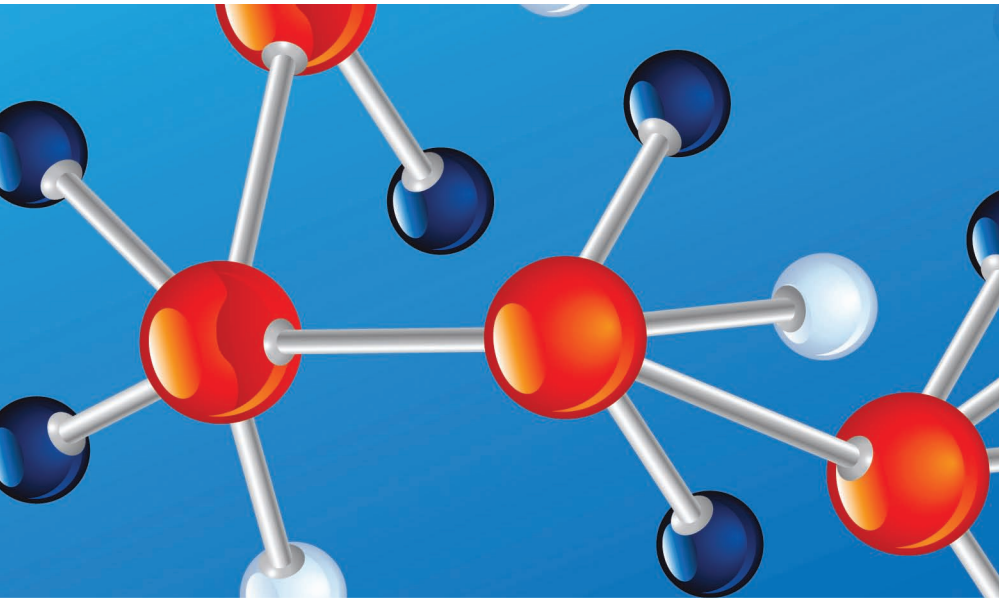
Update on Blood Brain Barrier



GAD65 ANTIBODIES, CHRONIC PSYCHOSIS, AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

Glutamic acid decarboxylase is the rate-limiting enzyme in the production of gamma aminobutyric acid, an inhibitory neurotransmitter. Autoantibodies to the glutamic acid decarboxylase 65 isoform have been associated with chronic psychotic disorders and are found in neurons and pancreatic islets. Blood samples were collected from normal controls (n=16), individuals with chronic psychosis with type 2 diabetes mellitus (n=3), and patients with chronic psychosis without diabetes (n=8). No differences were found between any of the groups for frequency of positive glutamic acid decarboxylase 65Ab samples (98th percentile of a healthy control group) or in mean values of glutamic acid decarboxylase 65Ab. Sample size was likely too small to detect differences if they do exist.

KEY WORDS

Glutamic acid decarboxylase, GAD65 autoantibodies, psychosis, diabetes, GAD65Ab

INTRODUCTION

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the production of gamma aminobutyric acid (GABA). There are two isoforms of GAD in humans, GAD65 and GAD67, each encoded by separate genes.[1–3] GAD65 predominates in pancreatic islets,[4] while both GAD65 and GAD67 are found in neurons.[5,6] Both can be detected in peripheral circulation indicating a compromise in the blood brain barrier (BBB), and have been associated with chronic psychotic disorders, though statistical significance was not reached. GAD65 and GAD67 autoantibodies have been associated with chronic psychotic disorders, though statistical significance was not reached.[7–9] GAD65 autoantibodies (GAD65Ab) have also been associated with type 1 diabetes and latent autoimmune diabetes in adults (LADA),[10–12] as is the case for GAD67 autoantibodies,[13,14] although the latter are believed to be caused by crossreactivity of GAD65Ab, rather than being specific for GAD67.

In a population of individuals with chronic psychotic disorders, we sought to determine whether higher levels of GAD65Ab were associated with type 2 diabetes mellitus (DM2).

METHODS

Blood samples were collected from three experimental groups: healthy controls (n=16), nondiabetic individuals with chronic psychosis (n=8), and patients with chronic psychosis and DM2 (n=3). Individuals with chronic psychotic disorders had a diagnosis of schizophrenia or schizoaffective disorder. Subjects with type 1 diabetes were excluded. Patients with tardive dyskinesia (TD)

were also excluded from the study (n=1) because Yarlagadda et al[9] found that GAD65Ab levels were markedly higher in individuals with chronic psychotic disorders and TD when compared to individuals with chronic psychotic disorders without TD and healthy controls. GAD65Ab indices of each sample were determined using a standard radioligand binding assay.[15] Cut-off value for GAD65Ab positivity was established as the 98th percentile of a healthy control group (n=50). We compared frequency of positive GAD65Ab samples in our three experimental groups. We also compared the mean values of GAD65Ab between our three experimental groups. Analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 17.

RESULTS

Frequency of GAD65Ab-positive samples in healthy controls and individuals with chronic psychotic disorders with and without DM2 is shown in Table 1. Using the Pearson Chi-Square test, we determined the differences in frequency of positive GAD65Ab samples between the three groups was not significant (p=0.28). The different frequencies of positive samples between individuals with chronic psychotic disorders with DM2 and individuals with chronic psychotic disorders without DM2 was not statistically significant using Fisher's exact test (p=0.27). The difference between healthy controls and individuals with chronic psychotic disorders without DM2 was not statistically significant (p=0.54). (Note: the sample size is too small for a Pearson Chi-Square test, which at a minimum usually requires 80% of the expected values to be >5).

Mean GAD65Ab index in healthy controls and individuals with chronic psychotic disorders with and without DM2 is shown in Table 2. We used ANOVA to determine that there was no statistically significant difference in mean GAD65Ab index between the three groups (p=0.77). T-tests showed no significant difference in mean GAD65Ab indices between individuals with chronic psychotic disorders with DM2 and individuals with chronic psychotic disorders without DM2 (p=0.99). T-tests similarly showed no significant difference in mean GAD65Ab indices between healthy controls and individuals with chronic psychotic disorders without DM2 (p=0.53).

DISCUSSION

There was no statistically significant difference in GAD65Ab levels between the three groups in our sample of healthy controls, nondiabetic individuals with chronic psychotic disorders, and individuals with chronic psychotic disorders and with DM2. The analysis was limited by the small number of individuals with chronic psychotic disorders enrolled in the study.

None of the samples tested positive for GAD67Ab, another possible marker of chronic psychotic disorders in association with type 1 diabetes.

We plan to repeat this study with several modifications. First, we will collect a larger number of subjects for each of the groups. Second, we will analyze the sera for S100 levels. S100 is a calcium-binding protein that is expressed at high levels and released by glial cells in the brain and is a possible marker of blood-brain barrier compromise.[16–18] We hypothesize that a compromise of the BBB (caused by infection or trauma) allows a temporary access of neuronal GAD65 to the periphery, initiating the formation of GAD65Ab. Likewise S100 will gain access to the periphery and will be detectable in the periphery. We expect S100 levels to be elevated in patients with chronic psychotic disorders and to correlate with elevated GAD65Ab levels. S100 may even prove to be a marker of chronic psychotic disorders, independent of GAD65Ab levels.

TABLE 1. GAD65Ab-positive samples

COHORTS	FREQUENCY
Controls	2/16 (12.5%)
Chronic psychotic disorders without DM2	0/8 (0%)
Chronic psychotic disorders with DM2	1/3 (33%)

DM2: type 2 diabetes mellitus

TABLE 2. Mean GAD65Ab index

COHORTS	INDEX
Controls	0.037
Chronic psychotic disorders without DM2	0.029
Chronic psychotic disorders with DM2	0.029

DM2: type 2 diabetes mellitus

CONCLUSION

Our present analysis of individuals with chronic psychotic disorders with and without DM2 did not reveal any difference regarding the presence of GAD65/67 autoantibodies. We acknowledge that our sample group is too small to allow this analysis and propose to re-analyze a substantially larger cohort.

This new cohort will be used to test our hypothesis that the reduced GABA

levels observed in chronic psychotic disorders are caused by GAD65/67 enzyme inhibition by the respective autoantibodies. We therefore propose to establish a correlation between GABA levels and the presence of GAD65/67 autoantibodies and characterize GAD65/67 autoantibodies for their epitope specificity and their capacity to inhibit enzyme activity.[19] Finally, we will also determine GAD65/67 expression levels in individuals with chronic psychotic disorders with and without DM2.

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